REMARKS

Claims 1, 2, 4-22, and 25-28 are pending in this application. Claims 1, 2, 4-16, and 25-28 are pending on the merits. Claim 17-22 have been withdrawn without prejudice as being directed to a non-elected species. Claims 3 and 23-24 have been canceled without prejudice. Applicants reserve the right to file one or more divisional, continuation, or continuation-in-part applications directed to any withdrawn or canceled subject matter.

New claims 30-34 have been added. Support for the new claims may be found throughout the application for example in Tables 1, 2 and 3.

No new matter has been added by the amendments.

I. The Rejections Under 35 U.S.C. § 103(a) Should be Withdrawn

Claims 1-2, 4-11, 16 and 28 are rejected under 35 U.S.C. 103(a) as allegedly obvious over U.S. Patent No. 5,705,366 to Backus *et al.* ("Backus") in view of Bustin *et al.,* Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193 ("Bustin") and further in view U.S. Patent No. 5,773,258 to Birch *et al.* ("Birch").

Claims 12-15 are rejected under 35 U.S.C. 103(a) as allegedly obvious over Backus in view of Bustin and Birch as applies to claims 1-2, 4-11, 16 and 28 and further in view of U.S. Patent No. 5,459,038 to Reed *et al.* and Demke *et al., Biotechniques*, 1992, vol. 12, no. 3, p. 333-334. Claim 25 is rejected under 35 U.S.C. 103(a) as allegedly obvious over Backus in view of Bustin and Birch as applied to claims 1-2, 4-11, 16 and 28 above, and further in view of U.S. Patent No. 6,183,998 to Ivanov et al. ("Ivanov"). Claims 26-27 are rejected under 35 U.S.C. 103(a) as allegedly obvious over Backus in view of Bustin and Birch as applied to claims 1-2, 4-11, 16 and 28 above and further in view of Mansfield et al., Molecular Cellular Probes, 1995, vol. 9, p. 145-156 ("Mansfield").

The claims encompass, *inter alia*, methods for coamplification of two or more target nucleic acids having different sequence compositions present at comparable copy numbers wherein the maximum difference between the lowest and highest copy number is 10-fold said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequently steps of: (A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature, T_1, for denaturation of the strands of the target

nucleic acids or their primer extension products, and (B) priming the denatured strands with a set of primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2, and (C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3, provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same, and wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight % of a nonionic, polymeric volume exclusion agent, a theremostable hot start DNA polymerase, and optionally a sequence specific labeled probe which binds with the primer binding regions and which is detectable after hybridization, and (D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle detecting one or more of the primer extension products as an indication of one or more of the target nucleic acids.

According to the office action, Backus discloses "a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume excluding agent." Office Action of May 22, 2009, p. 2, lines 18-19. The Office concedes, however, that "neither Backus nor Birch explicitly teach that the two or more target nucleic acids are present in comparable copy numbers and the highest copy number is 10 fold." Office Action of May 22, 2009, p. 7, lines 16-18. However, the Examiner argues that Bustin teaches, "the coamplification of nucleic acids which are present at comparable copy numbers, wherein the maximum difference between the lowest and highest copy number is 10-fold..." Office Action of May 22, 2009, p. 8, lines 4-6.

The Examiner concludes that, "one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number." Office Action of May 22, 2009, p. 9, lines 11-15.

Applicants respectfully disagree and traverse the rejection for at least the following reasons.

A. The Combination of Backus and Bustin Fails to Support a Case of *Prima Facie* Obviousness as it Provides No Reasonable Expectation of Success

The Federal Circuit recently reviewed the standard for obviousness under 35 U.S.C. § 103, in *In re Kubin* (Fed. Cir. 2009), 2008-1184, taking into account the Supreme Court's decision in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007). The Court specifically addressed the situation in which an invention that is "obvious to try" nevertheless remains patentable in view of *KSR*.

The Court stated that in, "such circumstances, where a defendant merely throws metaphorical darts at a board filled with combinatorial prior art possibilities, courts should not succumb to hindsight claims of obviousness." *Kubin* at 14. The Court concluded that, "the inverse of this proposition is succinctly encapsulated by the Supreme Court's statement in *KSR* that where a skilled artisan merely pursues 'known options' from a 'finite number of identified, predictable solutions,' obviousness under § 103 arises." *Id.*

The Federal Circuit reiterated its previous holding in *O'Farrell* as consistent with *KSR* stating that, "an obviousness finding was appropriate where the prior art 'contained detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting that it would be successful.'" *Kubin* citing *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). The Court further quoted *O'Farrell* for the rule that, "[o]bviousness does not require absolute predictability of success . . . all that is required is a reasonable expectation of success." *Id.*

Thus, an invention that is "obvious to try" nevertheless remains patentable if the cited prior art combination fails to provide one of skill in the art (at the time of filing of the relevant application) with "a reasonable expectation of success." Applicants respectfully assert that the Examiner is combining elements from Bustin and Backus and erroneously suggesting that the combination would provide the skilled artisan in 2002 with a reasonable expectation of success.

The claimed subject matter relates to the design and execution of multiplex polymerase chain reactions. Multiplex polymerase chain reaction ("PCR") is a variant of PCR in which two or more target sequences can be amplified by including more than one pair of primers in the same reaction. At the time of filing of the present application, selection and optimization of multiplex PCR reaction primers, reagents, conditions and parameters was anything but a straight forward undertaking.

In a 2002 multiplex PCR review article by Markoulatos *et al.* in Journal of Clinical Laboratory Analysis 16:47–51 (2002); the authors indicated that "common problems encountered in multiplex PCR [are] spurious amplification products, uneven or no amplification of some target sequences, and difficulties in reproducing some results…" Abstract. As such, the authors cautioned that "development of an efficient multiplex PCR usually requires strategic planning and multiple attempts to optimize reaction conditions." Abstract. Markoulatos *et al.*, indicate that,

> the relative concentration of the primers, concentration of the PCR buffer, balance between the magnesium chloride and deoxynucleotide concentrations, cycling temperatures, and amount of template DNA and Taq DNA polymerase are important. An optimal combination of annealing temperature and buffer concentration is *essential* in multiplex PCR to obtain highly specific amplification products.

[Emphasis added] Abstract. Of key importance to the current discussion is, Markoulatos *et al.*'s statement that the "list of various factors that can influence the reaction is *by no means complete*." [Emphasis added] Abstract. Indeed, Bustin similarly concludes that, "successful multiplexing is *never* trivial and *requires* careful consideration about the suitability of both chemistry and instrumentation." [emphasis added], Bustin at 185, left col. second paragraph.

Backus touts the ability to use the limiting primer approach to optimizing multiplex PCR as a result of its use of a volume exclusion agent. Backus at Col. 4:30-35. However, this also fails to provide the skilled artisan any meaningful guidance.

The "User Bulletin #5" for the ABI Prism 7700 Sequence Detection System, August 10, 1998 (updated 01/2001) (the "Bulletin"), describes the limitations of the "rate limiting primer" protocol suggested as desirable by Backus. It specifically states on page 5, that the limiting primer method, "does not guaranty successful determination of limiting primer concentrations for all assays." The two solutions provided by the Bulletin to react to any problems with the limiting primer method are a running of the "reactions in separate tubes… [so as one reaction can] proceed unaffected by the other" or a "[r]edesign and retest of primers." Bulletin at page 5.

The Bulletin in effect, therefore, states that should problems arise in the limiting primer approach; the technician should either jettison the multiplex method or start from scratch with new primer design. The 2001 Bulletin clearly implies that that multiplex optimization *is simply not possible* under some conditions.

With respect to Bustin, the Examiner indicates that it, "clearly suggests that multiplex amplification is more successful when levels of mRNA are similar." Office Action of May 22, 2009, p. 19, lines 18-20. However, a careful review of Bustin would not further assist the skilled artisan. Bustin teaches the "simultaneous" amplification "with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized." Bustin further states that, "the ideal internal standard should be expressed at a constant level among different tissues of an organism … and should be unaffected by experimental treatment." Bustin at p. 182, right column.

Notwithstanding Bustin's idealized and theoretical "internal standard," Bustin fails to provide any guidance as to how to actually conduct multiplex PCR in such a context. Bustin merely cites Karge *et al.,* Methods of Molecular Biology, Vol. 110, Lipoprotein Protocols, J.M. Ordovas, Humana Press, Inc., Totowa, N.J., which describes the coamplification of a test RNA and synthetic longer length RNA control template. Karge *et al.*, spiked the reaction mixture with a known amount of the RNA control template which was designed to be coamplified utilizing the *same* primers as the test RNA. Such methodology clearly aims to avoid the potentially endless experimentation associated with designing a reaction for amplifying multiple target nucleic acids using multiple primer pairs described by Markoulatos and in the Bulletin.

Therefore in 2002, the skilled artisan would had to go down the *never* trivial path of subtly adjusting a large number of *known* variables including concentration, sequence and GC-content of primers, concentration of Taq DNA polymerase, target template, PCR buffer, magnesium chloride, cycling lengths and temperatures and template. However, even more daunting for the skilled artisan would have been the *unknown* variables. Given that Markoulatos' list was "by no means complete," it would have often been impossible to determine whether an experimental modification of a variable was something that could be checked off of a finite list of potential solutions or whether the same potentially effective modification rendered the overall reaction inoperable through some other unknown factor. As such, the technician would not know whether they were approaching an optimization breakthrough or whether they should just give up on the multiplex approach or start from scratch as suggested by the Bulletin.

Accordingly, the design of any multiplex PCR assay designed to amplify two or more different target nucleic acids present at comparable copy numbers amounted to throwing metaphorical darts at a board filled with combinatorial prior art possibilities. It would be

mischaracterizing the state of art in 2002 to suggest that in developing the claimed broadly applicable multiplex PCR methodologies, a skilled artisan merely pursued "known options" from a "finite number of identified, predictable solutions." Again, there were so many unknown variables that a key 2002 multiplex review article conceded that its extensive list of potential solutions and variables was, "by no means complete." Markoulatos *et al.*, Abstract.

Applicants respectfully assert that the Examiner has provided insufficient justification as to how the skilled artisan would have derived a reasonable expectation of success in addressing these variables, from Backus' disclosure of the use of a volume exclusion agent. Backus merely indicates that the,

> presence of this agent effectively allows the user to reduce the amount of primer needed for efficient amplification of the nucleic acids, which reduction then allows manipulation of the procedure so one nucleic acid is amplified preferentially. Thus, *the primer can then be used as a rate limiting reactant.*

[Emphasis added] Backus at Col. 4:30-35. Further Backus states, "the volume exclusion agent also increases the rate of renaturation of the amplification reaction products which further reduces amplification efficiency for high copy target nucleic acids compared to the low copy target nucleic acids." Backus at Col. 4:36-40.

Backus therefore teaches that a volume excluding agent: 1) reduces the amount of primer needed for amplification; 2) allows for preferential amplification; 3) reduces amplification efficiency of high copy target nucleic acids compared to the low copy target nucleic acids. All of these conclusions go to the utility of volume exclusion agents in the amplification of target nucleic acids present at largely *variable* copy numbers. However, under *KSR*, "it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish *prima facie* obviousness of a new claimed compound." *Takeda Chem. Indus., Ltd. v. Alphapharm Pty., Ltd.*, 492 F.3d 1350, 1357 (Fed. Cir. 2007).

The Examiner states Backus, "the technique of including the volume-exclusion agent allows flexibility in amplification design and does not in any way indicate that the method is only applicable to targets that are present 100,000 fold difference in concentration." Office Action of May 22, 2009, at p. 19, lines 10-13. However, the Examiner has nevertheless proffered insufficient rationale as to why the skilled artisan would feel these characteristics *would* also be

useful in amplifying two or more different target nucleic acids present at *comparable* copy numbers.

In fact, there appears to be no evidence to suggest that the skilled artisan would have deemed the presence or absence of volume exclusion agents to effect the amplification of two or more different target nucleic acids present at *comparable* copy numbers any more or less than any other adjuvant (e.g., DMSO, glycerol, formamide, betain, BSA, etc.) or modulation of any other variable such as concentration, sequence and GC-content of primers or concentration of Taq DNA polymerase, target template, PCR buffer, magnesium chloride, cycling lengths and temperatures and template.

Therefore, without the having some specific reason as to why the skilled artisan would have employed a volume exclusion agent in the amplification of two or more different target nucleic acids present at *comparable* copy numbers, the skilled artisan would have deemed such an inclusion the equivalent of tossing another dart at the metaphorical multiplex PCR dartboard covered with combinatorial prior art possibilities. *Kubin* at14.

If anything, Backus' suggestion that volume exclusion agents reduce amplification efficiency **teaches away** from the use of volume exclusion agents in attempting the amplification of two or more different target nucleic acids present at *comparable* copy numbers. Backus at Col. 4:30-40. The skilled artisan would in fact, have been dissuaded from using an agent that reduces amplification efficiency in attempting the amplification of two or more different target nucleic acids present at *comparable* copy numbers. *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994).

In summary, the skilled artisan would not have had a reasonable expectation of success that the use of a volume exclusion agent would have provided a reasonable expectation of success in the amplification of two or more different target nucleic acids present at *comparable* copy numbers.

B. The Claimed Methods Provide Unexpectedly Superior Results

Even if the Office were to have made a *prima facie* case of obviousness, the claimed methods provide results that are unexpectedly superior to those that could have reasonably been expected. Applicants respectfully assert that the experimental evidence in the subject application

of unexpectedly superior results is sufficient to rebut any colorable case of *prima facie* obviousness.

A *prima facie* showing of obviousness may be rebutted by demonstrating "that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995).

Bustin states that multiplex PCR is limited by mutual interference of multiple sets of PCR primers, which can reduce the dynamic range of the sensitivity and make quantification unreliable." Bustin at p. 185, left column. As such, Bustin concludes that, "if quantification is the main aim, it is probably best to limit multiplexing to the detection of *two* or *three* transcripts." Bustin at 185, right col. second paragraph. Indeed, Backus only discloses the co-amplification of two independent target nucleic acids.

The Applicants also appreciated these problems when they indicated that the presence of many different primers leads to a high probability of primer dimer formation. Applicants stated that, "[t]he presence of primer dimers dramatically reduces the efficiency of the reaction." at paragraph 0021. Applicants further indicate that, "[e]fficient co-amplification of multiple targets (multiplex PCR) is only possible when reaction conditions are chosen that allow all reactions to take place simultaneously and all reactions only minimally influence each other." Application at paragraph 22.

In addition to designing primers that will minimize the formation of primer dimers, Markoulatos *et al.*, states that "in multiplex PCR, adjusting primer amount for each locus is also essential." [Emphasis added]. This technique of "primer biasing" in multiplex PCR is referred to in paragraph 15 of the current application and is described as "very difficult to perform" in paragraph 23.

In light of the Bulletin and the Backus, Bustin and Markoulatus references, the skilled artisan would have believed that a fair amount of luck and an enormous amount of optimization over a *long and incomplete* list of variables, conditions and parameters would be required order to develop a new multiplex PCR assay. The skilled artisan would have been intimidated enough by the thought of attempting to design a multiplex PCR capable of amplifying two or more different target nucleic acids present at *comparable* copy numbers. However, the critical point here is that they would also have clearly understood that the amount of luck and optimization

required to amplify two targets *would increase exponentially* with the number of additional distinct amplification primer pairs present in the desired multiplex assay. That is precisely why Bustin warned the scientific public that, "it is probably best to limit multiplexing to the detection of two or three transcripts." Bustin at 185, right col. second paragraph.

Applicants respectfully assert that their invention unexpectedly bridged these substantial technical hurdles. Applicants discovered a rapid and efficient method that allows the skilled artisan to conduct a multiplex PCR assay in which at least six (6) if not eight (8), target sequences could be simultaneously amplified with little or no optimization work required. See Tables 2 and 3 of the subject Application, respectively.

The skilled artisan would have been surprised that the simple but elegant combination of a hot-start DNA polymerase and a volume exclusion agent (previously thought only useful in the presence strong differences in target copy numbers) is sufficient to obviate the need to: 1) invent primers and reaction conditions that allow all PCR reactions to take place simultaneously only minimally influencing each other (Application at paragraph 22); and 2) embark on a non-trivial path of tail-chasing optimization experiments, such as the "very difficult to perform" primer biasing experiments (*Id* at paragraph 23) and the potentially inconclusive Limiting Primer Protocols described in the Bulletin. All of these would have been deemed necessary to achieving the Applicants' disclosed multiplex results.

Clearly the claimed methods demonstrate a superior advantage that the person of ordinary skill in the relevant art would have found surprising or unexpected in 2002. *Soni* at 750. Accordingly, the Applicants respectfully assert that the subject Application provides unexpectedly superior results that are sufficient rebut any *prima facia* case of obviousness. *Id.*

Based on at least the arguments set forth above, Applicants respectfully request that the rejection of the claims under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

II. Conclusion

Applicants believe that claims 1, 2, 4-16 and 25-28 are allowable and respectfully request allowance thereof. The Examiner is invited to telephone the undersigned if that would be helpful to resolving any issues.

It is believed no fees are due; however, the commissioner is authorized to charge any fees and credit any overpayments to Deposit Account No. 50-2957 which may be due.

Respectfully submitted,

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By: Thomas A. Haag, Ph.D., Esq.
Reg. No. 47,621

Fanelli Strain & Haag PLLC
Customer No. 0094136
1455 Pennsylvania Avenue, N.W.
Suite 400
Washington, D.C. 20004
Telephone: 202.621.1840
Facsimile: 202.621.1844